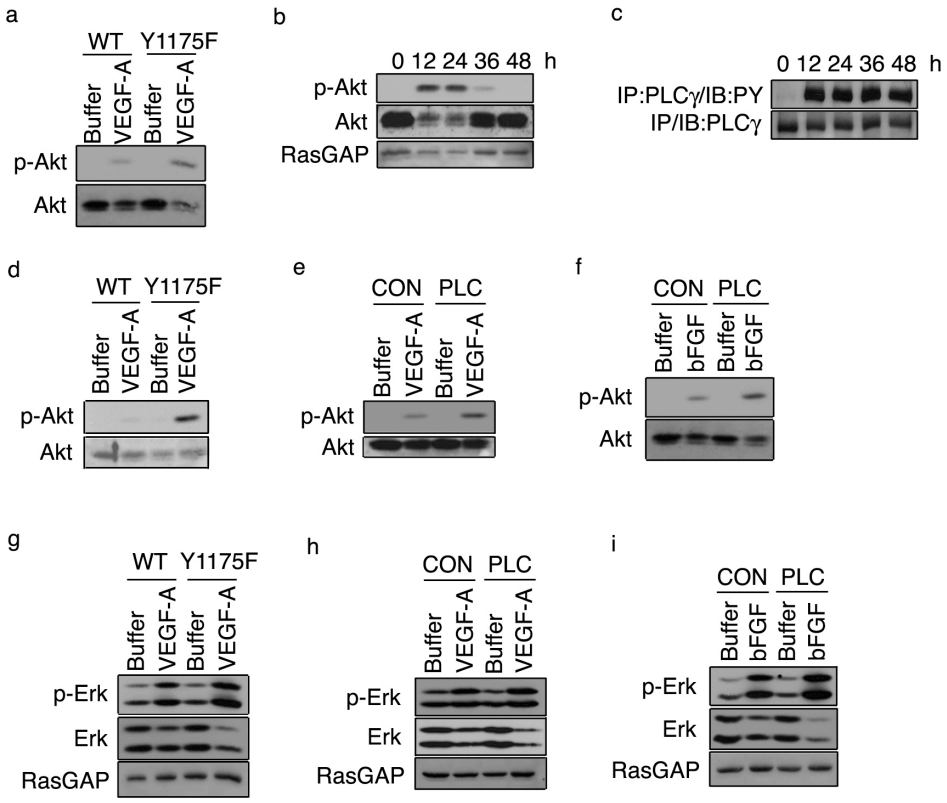
Figure S3. (a) Activation of Akt was increased in the cells expressing a VEGFR2 mutant that was unable to activate PLCγ. Monolayers of BRECs expressing WT and Y1175F receptors were stimulated with 25 ng/ml VEGF-A for 2 min. The cells were harvested and total cell lysates were subjected to a Western blot using an anti-phosphoAkt (Ser473) antibody. The blot was then stripped and re-probed with an anti-Akt antibody. **(b) A decline in phosphoAkt occurred as tubes regressed.** BRECs were organized into tubes. At the desired times total cell lysates were made and subjected to Western blot analysis using anti-phosphoAkt (Ser473) and anti-Akt antibodies. The blot was stripped and reprobed with RasGAP as a loading control. **(c) Phosphotyrosine of PLCγ did not change when tubes regressed.** BRECs were subjected to a tube assay. At the indicated times total cell lysates were made, immunoprecipitated with a PLCγ antibody, and the phosphotyrosine content of PLCγ was assessed by Western blot analysis using an anti-phosphotyrosine antibody (top panel). The bottom panel was generated by re-probing the blot with an anti-PLCγ antibody. **(d)Akt was activated to a greater extent in Y1175F-expressing cells.** BRECs expressing the indicated receptors were subjected to a tube assay supplemented with 2.5 ng/ml VEGF-A. At day 2 total cell lysates were made and subjected to Western blot analysis using anti-phosphoAkt (Ser473) and anti-Akt antibodies. **(e) Attenuating PLCγ increased activation of Akt in response to VEGF-A.** HUVECs were transfected with either siRNA for PLCγ (PLC) or non-targeting control (CON) and subjected to a tube assay in the presence of 2.5 ng/ml VEGF-A. At day 2 total cell lysates were made and subjected to Western blot analysis using anti-phosphoAkt (Ser473) and anti-Akt antibodies. **(f) Attenuating PLCγ increased activation of Akt in response to bFGF.** Same as panel e, except using bFGF in place of VEGF-A. **(g, h, i) Erk activation was unimpaired by interfering with PLCγ activation or expression.** In panel g BRECs expressing the indicated receptors were subjected to a tube assay and supplemented with 2.5 ng/ml VEGF-A. At day 2 total cell lysates were made and subjected to Western blot analysis using anti-phospho-Erk and anti-Erk antibodies. In panel h HUVECs were transfected with either siRNA for PLCγ (PLC) or non-targeting control (CON) siRNA and subjected to a tube assay in the presence of 2.5 ng/ml VEGF-A. At day 2 total cell lysates were made and subjected to Western blot analysis using anti-phospho-Erk and anti-Erk antibodies. Panel i is the same as panel h, expect that bFGF was used in place of VEGF-A.

Supplementary Materials and Methods

Antibodies. Rabbit polyclonal anti-phospho-p44/42 MAP kinase antibody, and anti-p44/42 MAP kinase antibody were obtained from Cell Signaling Technology.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